REMARKS/ARGUMENTS

Claims 66-71, 73-75 and 77-85 and 87-100 are active in this case.

Support for the amendment to Claim 66 and newly added Claims 99-100 is found on page 10, 1st, 2nd and 3rd paragraphs; page 15, 1st ¶, and the Examples on pages 23-24. The fact that the cells so cultured have enhanced cytokine secretion is disclosed on page 11, lines 6-7 and pages 23-26.

No new matter is believed to have been added by the amendments.

The claims of this application are directed to methods of obtaining human T cells with enhanced replicative function and cytokine secretion. Those cells are cultured under certain conditions as defined in the claims. As discussed in the specification, an advantage of the present invention is the discovery that culturing cells, including T-cells, one can obtain a population of cells with enhanced replicative function and other functions such as cytokine secretion making these cells particularly useful for therapeutic applications--noting that therapeutic uses of cultured T-cells is known in the relevant field, something already acknowledged by the Office.

The rejection under 35 USC 103(a) in view of the '358 patent combined with Stacey is not tenable because:

(A) While Stacey does describe replacing medium (at pages 21 and 24), Stacey does not describe replacing medium at a rate of at least 25% daily replacement continuously for more than one day and for a time sufficient to obtain T-cells with enhanced replicative potential (and cytokine release) and wherein the cell density of the T-cells is not substantially reduced or adjusted at any time during the culturing. Further, Stacey does not

describe a starting innoculum of cells with an expansion of cells as set forth in Claims 99 and 100 under these conditions. Therefore, the art cited does not teach or suggest all of the limitations of the claims.

(B) Even presuming that the '358 patent with Stacey did at least suggest all of the limitations (based on the "routine experimentation" alleged in the Action at page 5), culturing T-cells under the conditions defined and then using those T-cells in immunotherapy is contrary to what the art teaches and yields more than the predictable result.

The standard for obviousness as explained by the United States Supreme Court in *KSR Intern. Co. v. Teleflex, Inc.*, 127 S.Ct. 1727, 1740 (2007) is, e.g.,:

> when a patent 'simply arranges old elements with each performing the same function it had been known to perform' and yields no more than one would expect from such an arrangement, the combination is obvious.

In the present situation, the combination of US '358 and Stacey cited by the Examiner does not provide the requisite predicable result because the art teaches different methodologies with a lack of expectation that the claimed compositions would have an "enhanced replicative potential" when cultured under the conditions defined (see also new claims 99 and 100). The conditions as defined in the claims was contrary to what the conventional wisdom was regarding culturing T-cells and further the findings of the cells cultured as defined in the claims was not predictable from that knowledge.

The results for T-cells as shown in the specification are unexpected and novel notwithstanding what US '358 and Stacey describe.

The '358 patent is relied upon for using T-cells in immunotherapy and the Stacey manual is relied upon for the general methodology of culturing cells (citing pages 21 and 24).

The '358 patent in col. 23 discusses culturing cells and replacing medium but teach that the cell density is to be maintained in a particular amount (e.g., 0.5 x 10^6/ml) --col. 23, lines 45-57. Thus, this means that during the entirety of the culture medium the cells must be substantially reduced or adjusted during the culturing because the cells are certain to expand in number during the culturing procedure.

This is also what Stacey describes on page 24 (Suspension cell cultures) --see step 4 in particular.

Stacey and the '358 patent describe removing cultured cells, diluting them in fresh medium to a reduced cell density and then reculturing the cells. Neither Stacey nor the '358 patent describe or otherwise suggest replacing medium at a rate of at least 25% daily replacement <u>continuously for more than one day without dilution or removal of a proportion of the culture</u>. At least on this basis, the rejection is untenable.

Further, what the '358 patent and Stacey describe is that which is conventional in the art. For example, T-cell concentrations for conventional processes in tissue culture flasks or gas permeable culture bags are maintained optimally at 10^5 to 10^6 per ml or less based on common knowledge in the field of cell culture. Conventional wisdom suggests that when T-cells achieve a maximum cell concentration of 1-3 million cells per ml, the cultures must be diluted and split immediately into multiple cultures such that density is restored to 5 x 10^5 cells/ml or less (i.e. "hemi-depletion" to maintain low cell density by adding medium and/or splitting individual cultures into multiple cultures). Typically, this process is repeated several times (generally every 2-7 days during the course of standard T-cell culture (See Riddell and Greenberg, 1990 *J Immunol Methods 128*:189-201 for one representative example of this approach for T-cells).

Thus, it is apparent that the art cited in the rejection teaches away from that which is claimed, e.g., "not substantially reduced or adjusted at any time during the culturing." (see MPEP § 2141.02: "prior art must be considered in its entirety, including disclosures that teach away from the claims").

The standard processes are costly, labor intensive and consist of multiple manual open-process steps which are difficult or impossible to implement for multiple samples under stringent clinical and regulatory requirements including current Good Manufacturing Practices (cGMP). These constraints are a major limitation to the widespread evaluation and implementation of adoptive T-cell based immunotherapies in large multi-center Phase II and Phase III clinical trials, potentially leading to widespread delivery and commercialization of therapy. The new capability to produce highly active T-cells in a single closed process without subculture dramatically reduce or eliminate open and manual steps compared to the conventional methods described in US '358, Stacey et al., and by other investigators thus improving efficiency, automation and safety for patients' therapy.

In contrast, as described in the Examples of the application (see discussion starting at page 23) T-cells can be inoculated at low density (10^3-10^5 /ml or less) and grown to high density (10-40 x 10^6 cells/ml) in a single continuous process without splitting or sub-culture using frequent medium exchange methodology as set forth in the claims. In addition, medium exchange can be ramped based on lactate production so as to maintain a lactate tolerance level of 0.5 – 1.0 mg/ml as determined by sampling and measurement in the waste medium.

Obtaining T-cells with enhanced proliferative potential and cytokine secretion under these continuous culture conditions is entirely unexpected and counter-intuitive to those skilled in the art as indicated above (i.e. conventional wisdom emphasizes splitting cultures to maintain low density). *KSR*, *supra*.

Here the applicants have made it clear that the methodology claimed was contrary to conventional wisdom for T-cells and what was obtained, in terms of enhanced proliferation and cytokine release, was entirely unexpected from the teachings of US '358 and that conventional wisdom exemplified in the Stacey manual.

The Applicants have observed unexpectedly enhanced activity as measured in such bioassays including cytokine release and proliferation compared to conventional low density hemi-depletion cultures.

Furthermore, T-cells derived in the examples proliferate more vigorously in secondary cultures (after culture in primary cultures to densities exceeding 10-40 x 10^6 per ml in a single bioreactor cassette) and also release cytokines at higher levels than T-cells cultured in parallel under conventional low density (<1-3 x 10^6 T-cells per ml) using hemi-depletion methods. Thus, continuous culture under perfusion conditions does not have deleterious effects on cell division and may prevent or minimize immunological senescence. This unexpected result when considering conventional culture (as highlighted above) forms the basis of the invention consisting of continuous low to high density T-cell expansion without subculture under medium perfusion conditions. This T-cell culture strategy and potential benefits on proliferative potential or cytokine release would not be obvious to one skilled in the art. In the attached Rule 132 Declaration, Dr. Smith provides further basis as to the unexpected nature of what is described.

Accordingly, withdrawal of this rejection is requested.

The rejection under 35 USC 112, first paragraph based on the allegation that the claims are not enabled is respectfully traversed.

By the Office's own admission (see the rejection citing the '358 patent above), the use of T-cells for immunotherapy was generally known. This is indeed consistent with what has

been explained previously by the Applicants. Notwithstanding this knowledge and the fact that the inventors have discovered a better way to obtain such cells for immunotherapy, the rejection alleges that the specification does not provide suitable basis to use these better T-cells for a known therapeutic method for at least the reason that only *in vitro* experiments are shown (see, e.g. ,page 4 of the Action).

As has been discussed previously, the invention is based on the discovery that culturing cells in the manner defined in the claims allows one to obtain cells that have significant capabilities in proliferating *ex vivo* and the cells obtained also have higher biological function, i.e., are more potent cells (referring to pages 5-6 of the specification). Because the cells which are cultured according to the conditions claimed are more potent, these cells have a far greater capacity to be used in therapeutic applications wherever such cells are used. In other words, there is a body of evidence that is known in the art of using T-cells for therapeutic purposes. The inventors have discovered a way to make these cells better and more potent for such therapeutic applications.

Further support for the claims is provided in the attached Rule 132 Declaration by one of the named inventors, Douglas Smith. In this Declaration, Dr. Smith provides a discussion for how one knows the correlation of T-cell proliferation potential, telomere length, persistence *in vivo*, tumor rejection and clinical response.

Dr. Smith also presents and explains additional data that demonstrate why the cells obtained by the claimed method would, indeed be useful for therapeutic applications as set forth in the claims.

Accordingly, it should be apparent that the specification as originally filed coupled with the knowledge available in the art at that time, enables the culturing and generation of human T-cells and their use as now claimed.

Withdrawal of this rejection is requested.

It is also noted that a new rejection was raised in the parent application of the present case, i.e., U.S. application serial no. 09/027,671 citing U.S. '126 with newly cited U.S. '700 as a basis to allege that the claims of that application, which are to culturing the T-cells in a manner similar to those in the claims here, would have been obvious. In the interest of trying to compact prosecution here, the reasons why that combination of cited art does not render the present claims obvious are provided below. (see also the attached Rule 132 Declaration filed in the parent application 09/027,671).

In short, by the Office's own admission in the Action of the parent application and as apparent from a careful reading of the cited art, neither publication describes or suggests the aspect of culturing T-cells. Indeed, the U.S. '126 patent is focused on culturing dendritic cells--i.e., not T-cells, and the U.S. '700 patent provides a teachings for culturing cells, generally, in the specialized apparatus that is the subject of that patent. In fact, the only mention of cells in the U.S. '700 patent is in column 2 where the patent describes that the method and apparatus can be used to culture any cell, bacterial, human or other. Bacterial cells and mammalian cells (such as transformed cells that are commonly cultured) have vastly different properties, require vastly different culture conditions and techniques and, in fact, it is well-known that even among cells of a particular species that what one knows about one cell type gives the researcher no *a priori* information as to how cells of a different type will behave in that culture. It is further worth noting that the U.S. '700 patent provides no working examples of actually culturing cells in its specialized apparatus, particularly not mammalian cells, nor T-cells.

Further, as explained in the previous reply supported by actual relevant literature for T-cells, the conventional process of culturing is to maintain T-cell concentrations in tissue

culture flasks or gas permeable culture bags at an optimal concentration of 10^5 to 10^6 per ml or less based on common knowledge in the field of cell culture. Conventional wisdom suggests that when T-cells achieve a maximum cell concentration of 1-3 million cells per ml, the cultures must be diluted and split immediately into multiple cultures such that density is restored to 5 x 10^5 cells/ml or less (i.e. "hemi-depletion" to maintain low cell density by adding medium and/or splitting individual cultures into multiple cultures). Typically, this process is repeated several times (generally every 2-7 days during the course of standard T-cell culture (See Riddell and Greenberg, 1990 *J Immunol Methods 128*:189-201, previously submitted, for one representative example of this approach for T-cells).

Thus, what was previously provided by the Applicants is, in fact, more relevant to the art of culturing T-cells than either of the cited U.S. '126 or U.S. '700 patents--reiterating that the teachings of those references are to dendritic cells or prophetically any cells, bacterial, yeast, mammalian, etc.

The Office cites the recent Supreme Court case in *KSR Intern. Co. v. Teleflex, Inc.*, 127 S.Ct. 1727, 1740 (2007) in support of the rejection. Applicants agree that this is the relevant law and was why it was cited in the Applicants previous reply. Based on that law, if known elements are combined to achieve the predictable outcome then the claim is obvious. Admittedly, culturing dendritic cells was known as evidenced by the cited US '126 patent, medium exchange without splitting cells to achieve high density cultures was prophetically described by the US '700 patent, and culturing T-cells was known (see the publications previously cited by the Applicants, one of which is Riddell and Greenberg, cited above).

That the cited art does not actually teach a method of culturing T-cells, that the actual relevant art teaches not to culture as claimed here, and that the Applicants have discovered that not only can T-cells be cultured in direct contrast to the conventional knowledge but that such cultured T-cells are better than would have been thought in such a unconventional T-cell

culturing process, provides an undeniable conclusion that what the claims set forth was entirely unpredictable. Following the law of *KSR* applied to the fact that the art teaching culturing T-cells taught not to do it the way that is claimed and the Applicants discovery that when T-cells are cultured as claimed yields something more than what would have been predicted demonstrates that the claims would not have been obvious.

Simply for reference again, it should be noted that the T-cells derived in the examples proliferate more vigorously in secondary cultures (after culture in primary cultures to densities exceeding 10-40 x 10^6 per ml in a single bioreactor cassette) and also release cytokines at higher levels than T-cells cultured in parallel under conventional low density (<1-3 x 10^6 T-cells per ml) using hemi-depletion methods. Thus, continuous culture under perfusion conditions does not have deleterious effects on cell division and may prevent or minimize immunological senescence. This unexpected result when considering conventional culture (as highlighted above) forms the basis of the invention consisting of continuous low to high density T-cell expansion without subculture under medium perfusion conditions. This T-cell culture strategy and potential benefits on proliferative potential or cytokine release would not be obvious to one skilled in the art.

Therefore, any such rejection should not be applicable in the present case as it is also not applicable in the parent application.

Finally, a Notice of Allowance indicating all claims have been allowed is requested.

Respectfully submitted,

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